

# Effect of 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin on the Biliary Excretion of Indocyanine Green in Rat

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Chlorinated dibenzodioxins have been found as contaminants of various technical chlorinated compounds such as 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), which are widely used in agriculture. The dioxin contaminants may be involved in various pathologic states resulting from exposure to technical chlorinated compounds (1, 2). Liver necrosis has been observed in the animals which were treated with derivatives of chlorophenol (3) or other chlorinated compounds (4), and it was suggested that liver necrosis-causing factors were present in these compounds. In view of the above observation, the present study was undertaken to investigate whether 2,3,7,8-tetrachloro-*p*-dibenzodioxin (TCDD) has any effect on the hepatobiliary function of rat. Biliary excretion of indocyanine green (ICG) was used as the index of function. The dye was chosen because it is completely and rapidly excreted by normal liver into the bile by an active process (5).

## Materials and Methods

Male random bred CD rats weighing 350–400 g were treated PO with a single dose of 25 µg/kg or 5 µg/kg TCDD in acetone and

corn oil. The controls received an equivalent volume of the vehicle. At day 1, 7, and 16 after treatment, the effect of TCDD on the bile flow and the biliary excretion of ICG was examined. Animals were first anesthetized with penobarbital Na (50 mg/kg) IP. Through an abdominal incision, renal pedicles were ligated, and the common bile duct was cannulated with a blunt 23-gauge hypodermic needle shaft attached to an 8-in. piece of PE 50 tubing. Bile was collected for a 20-min period, and the amount collected was measured by weighing. At the end of 20 min, freshly prepared indocyanine green in aqueous solvent (Hynson, Westcott and Dunning, Inc.) was injected at a dose of 6.25 mg/kg into the femoral vein, and the bile was collected for another 20 min. The body temperature was monitored with a telethermometer and was maintained at 37° C by warming with an incandescent lamp. At the end of the experiment, blood was withdrawn by heart puncture and the liver was excised. The ICG concentration in bile, plasma, and liver was determined by measuring its absorption at 805 nm. The rate of ICG disappearance from plasma was also determined by measuring the dye concentrations in a series of plasma samples which were withdrawn by heart puncture 1, 2, 5, 8, 12, and 20 min after ICG injection from groups of control and TCDD-treated rats.

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## Results and Discussion

Bile flow during the first 20 min of the experiment increased after TCDD treatment as shown in Figure 1. The initial flow rate continued to increase through the 16th day, and rats receiving 25  $\mu\text{g/kg}$  TCDD had higher flow rates than those receiving 5  $\mu\text{g/kg}$  TCDD.

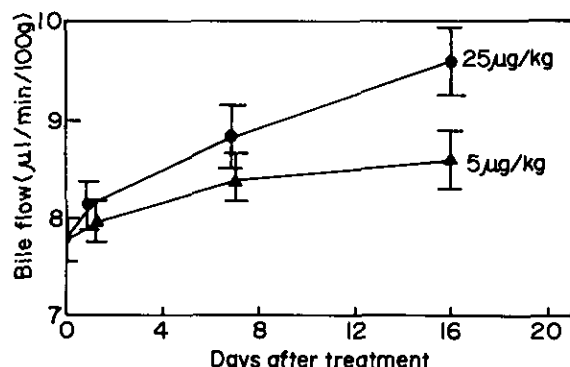


FIGURE 1. Effect of TCDD on bile flow. Each point is the mean  $\pm$  SEM of four animals. Difference from control is significant ( $P < 0.005$ ) for the 7th and 16th day after 25  $\mu\text{g/kg}$  TCDD treatment and 16th day after 5  $\mu\text{g/kg}$  TCDD treatment.

The increase in bile flow could be due to the increasing secretion of water by the hepatic cell or due to the decreasing reabsorption of water along the bile duct. Both mechanisms were possible, but further investigation will be needed for clarification.

TCDD also caused an increase in liver weight as shown in Figure 2. The increase in liver weight as expressed in grams liver weight per 100 g body weight was also dose-related since the higher dose caused a greater weight increase. Whether this increase in liver weight has any effect on the bile flow is not known. Similar results of bile flow increase and liver weight increase were observed in rat after phenobarbital treatment (6), and both TCDD and phenobarbital are potent microsomal enzyme inducers.

ICG excretion was also affected by TCDD treatment. The total amount of ICG excreted during 20 min after injection of the dye was

decreased significantly as shown in Figure 3. Less hepatic excretion of the ICG was observed with the larger dose of TCDD than with the smaller dose. The rate of excretion of this dye was still markedly suppressed by the 16th day after treatment.

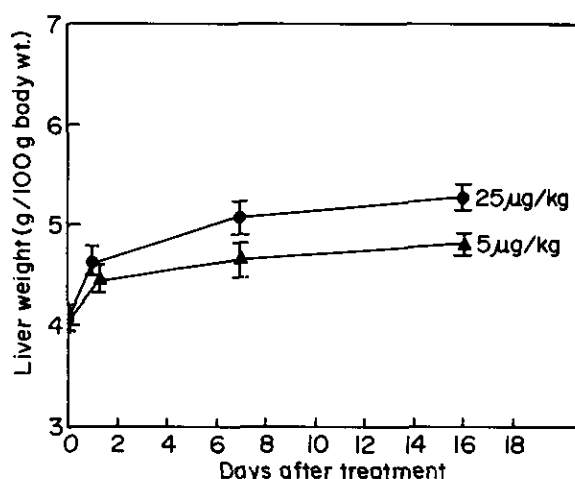


FIGURE 2. Liver weight of rat after TCDD treatment. Each point is the mean  $\pm$  SEM of four animals. The difference is significant ( $P < 0.05$ ) for the 7th and 16th day after treatment of either 5  $\mu\text{g/kg}$  or 25  $\mu\text{g/kg}$  TCDD.

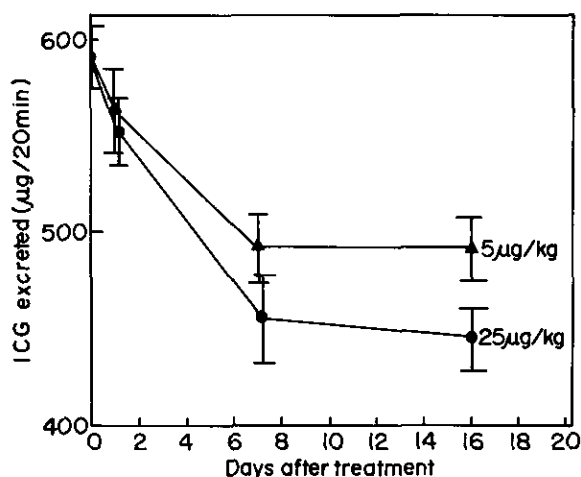


FIGURE 3. Effect of TCDD on ICG biliary excretion. The dose of ICG was 6.25 mg/kg, and the bile was collected for 20 min after ICG injection. Each point is the mean  $\pm$  SEM of four animals. The difference from control is significant ( $P < 0.05$ ) for the 7th and 16th day after treatment by either 5  $\mu\text{g/kg}$  or 25  $\mu\text{g/kg}$  of TCDD.

Table 1. Effect of TCDD on liver uptake and biliary excretion of ICG.

Time after TCDD treatment, days	ICG concentration in plasma, $\mu\text{g/ml}$ <sup>a</sup>		ICG concentration in liver, $\mu\text{g/g}$ <sup>a</sup>		ICG concentration in bile, $\mu\text{g/ml}$ <sup>a</sup>	
	TCDD 5 $\mu\text{g/kg}$	TCDD 25 $\mu\text{g/kg}$	TCDD 5 $\mu\text{g/kg}$	TCDD 25 $\mu\text{g/kg}$	TCDD 5 $\mu\text{g/kg}$	TCDD 25 $\mu\text{g/kg}$
Control	1.0 $\pm$ 0.09	1.9 $\pm$ 0.09	28.5 $\pm$ 0.9	28.5 $\pm$ 0.9	1130 $\pm$ 83	1130 $\pm$ 83
1 day	2.3 $\pm$ 0.10	2.75 $\pm$ 0.29	29.5 $\pm$ 2.3	29.0 $\pm$ 1.8	1015 $\pm$ 79	999 $\pm$ 64
7 days	3.0 $\pm$ 0.23	3.45 $\pm$ 0.15	37.5 $\pm$ 3.6	31.0 $\pm$ 2.1	869 $\pm$ 66	710 $\pm$ 45
16 days	3.1 $\pm$ 0.46	3.90 $\pm$ 0.58	41.0 $\pm$ 5.4	35.0 $\pm$ 3.6	860 $\pm$ 59	624 $\pm$ 46

<sup>a</sup> Dose of ICG was 6.25 mg/kg. Concentrations were determined 20 min after ICG injection. Each value is the mean  $\pm$  SEM from four animals.

The concentrations of ICG in plasma, liver, and bile were also analyzed separately for each animal 20 min after dye injection. The results (Table 1) showed that concentration of ICG in bile of TCDD-treated rat was lower than that of the control, and the ICG levels in plasma and liver of TCDD-treated rats were higher than that of the control. A greater depression of ICG concentration in bile and a greater retention of ICG in blood were caused by 25  $\mu\text{g/kg}$  dose of TCDD. In contrast, animals treated with 5  $\mu\text{g/kg}$  TCDD accumulated more ICG in liver than the animals treated with 25  $\mu\text{g/kg}$  TCDD. No sign of recovery was shown by the 16th day after treatment.

Bile-to-plasma, bile-to-liver and liver-to-plasma concentration ratios of ICG were also separately calculated and compared, as shown in Figure 4. Bile-to-plasma and bile-to-liver ratios decreased after TCDD treatment, and the higher dose decreased these ratios even further. Liver-to-plasma ratio decreased only after 25  $\mu\text{g/kg}$  but not after 5  $\mu\text{g/kg}$  TCDD pretreatment. TCDD appeared to inhibit both ICG uptake by the hepatic cell and the active secretion of ICG by the hepatic cell. Inhibition of both steps could result in the decreased total ICG excretion and lowered bile-to-plasma and bile-to-liver ICG concentration ratios as found.

The excretion of ICG from hepatic cell to bile was probably inhibited to a similar extent by both 25  $\mu\text{g/kg}$  and 5  $\mu\text{g/kg}$  TCDD. However, the inhibition of ICG uptake by the hepatic cell might be more dose-dependent; the inhibition was greater with 25  $\mu\text{g/kg}$  than with 5  $\mu\text{g/kg}$  TCDD, so the liver accumulated less ICG after 25  $\mu\text{g/kg}$  TCDD

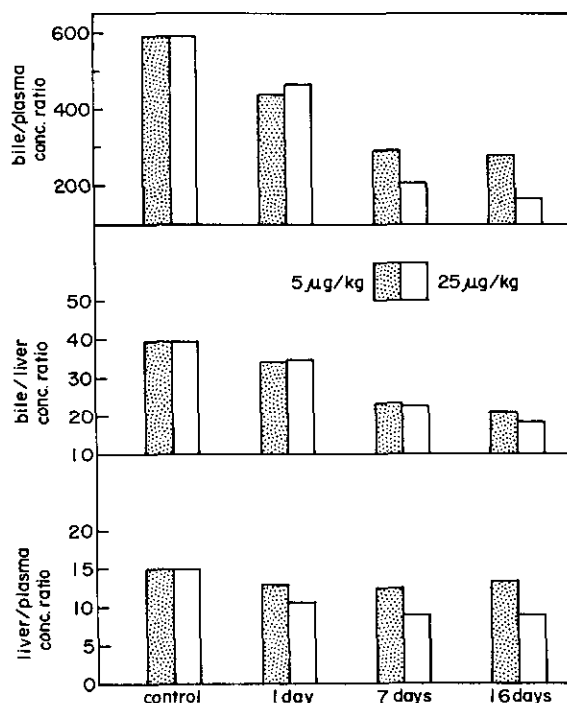


FIGURE 4. Change of ICG concentration ratios after TCDD treatment. Each bar represents the mean of four animals. The difference from control is significant ( $P < 0.05$ ) for all ratios at 7th and 16th day after TCDD treatment except the liver/plasma (L/P) ratios after 5  $\mu\text{g/kg}$  TCDD treatment.

treatment, and liver-to-plasma ICG concentration ratios decreased in animals treated with 25  $\mu\text{g/kg}$  but not 5  $\mu\text{g/kg}$  TCDD. Control animals should take up ICG into liver faster than TCDD-treated animals, but the secretion from liver to bile was even faster compared to the treated animals, so the control animals accumulated less ICG in both plasma and liver.

The rate of disappearance of ICG in plasma decreased after TCDD treatment, as shown in Figure 5. The 25  $\mu\text{g}/\text{kg}$  dose gave a greater reduction of disappearance rate than did the 5  $\mu\text{g}/\text{kg}$  dose, and the rate was lower

at the 16th day than at the 7th day after treatment. The decreasing rate of ICG disappearance in plasma gave further evidence that ICG excretion was damaged by the TCDD treatment.

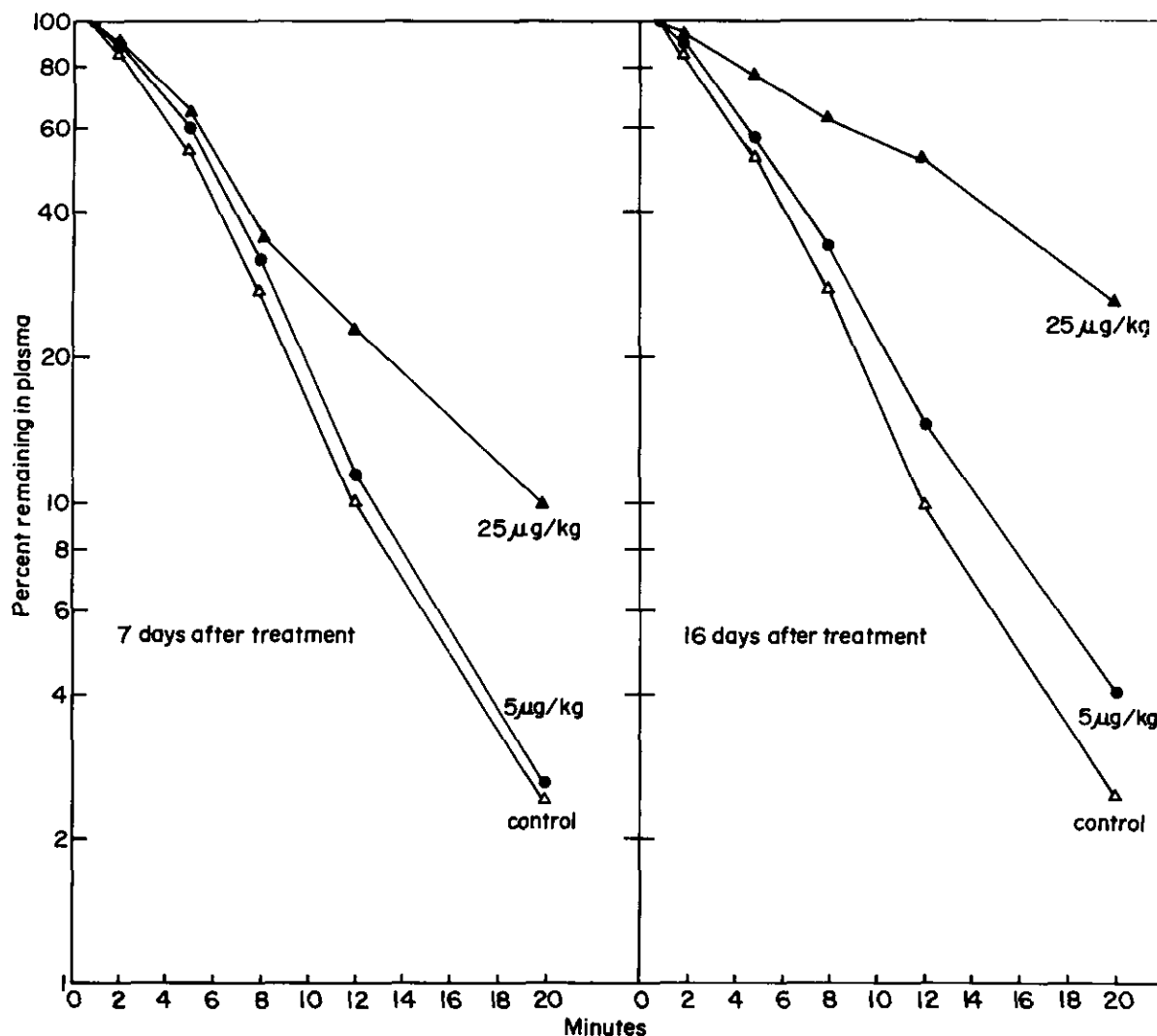


FIGURE 5. Effect of TCDD on the ICG disappearance rate in plasma. Each point is the mean of three rats.

## Summary

From the above observations, it was concluded that TCDD inhibited hepatobiliary excretion of ICG, and the inhibitory effect appeared to be a long-lasting one. Many anionic compounds, including endogenous substances such as bilirubin, are actively se-

creted through the similar mechanism by the hepatobiliary system. The decreased ICG excretory ability might also apply to other anionic compounds, and the etiology of reported cases of jaundice and porphyria after exposure to TCDD might be partly accounted for by the decreased biliary excretory ability.

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